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We have designed and synthesized a novel compound (11β) that efficiently triggers apoptosis in prostate cancer cells such as LNCaP. This bifunctional compound was designed to form DNA adducts that are camouflaged by the androgen receptor making them less readily repaired in AR+ prostate cancer cells. The aims of our studies are to investigate the mechanisms by which 11β is able to trigger apoptosis in target cells. One approach we are taking is to prepare structural analogs of 11β that have increased or decreased abilities to cause apoptosis in LNCaP cells. Methods have been developed that will permit us to determine the fates of 11β-DNA adducts in treated cells. Another objective is to identify the signaling events that lead from DNA adducts to activation of the apoptotic program. Finally we have begun to obtain encouraging results from animal experiments that indicate that molecules such as 11β may have clinical potential for the treatment of human tumors.

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INTRODUCTION

The objective of our research is to develop more effective therapeutics for the treatment of prostate cancers. One novel bifunctional compound (**11β**) that we have prepared rapidly induces apoptosis in several prostate cancer cell lines in vitro. The **11β** compound contains a chemically reactive nitrogen mustard linked to a steroid moiety that binds with high affinity to the androgen and progesterone receptor proteins. This compound was designed to create DNA adducts that form tight complexes with these steroid receptors that make the adducts difficult to repair in prostate cancer cells. Preliminary studies of **11β** in cell culture indicated that its effects on prostate cancer cells were different from those of other alkylating agents used in chemotherapy. The apoptotic responses of prostate cancer cells suggested that the **11β** compound might be a useful agent for chemotherapy. The Specific Aims of our research are to understand the fate of **11β**-DNA adducts in treated cells and investigate the mechanisms that lead to apoptosis. We also proposed experiments to assess the antitumor potential of **11β** in animal models of human prostate cancer.

BODY

Task 1: *Chemical synthesis of monochloro and 17-α-methyl analogs of our lead compound, 11β-(17αOH-estradien-4(5),9(10)-3-one)-C6NC2-mustard (**11β**), and assessment of their toxicity in prostate cancer cells in vitro.*

During the past year we have accomplished the synthesis the monochloro 11β analog. Substitution of a methoxy group for one of the chlorines of the aniline mustard was accomplished by the synthetic scheme that was presented in Fig 7 of our original proposal. The precurssor, [4-(3-Amino-propyl)-phenyl]-(2-chloro-ethyl)-(2-methoxy-ethyl)-amine was incorporated into the 11β molecule, producing the monochloro 11β compound shown in Fig 1.

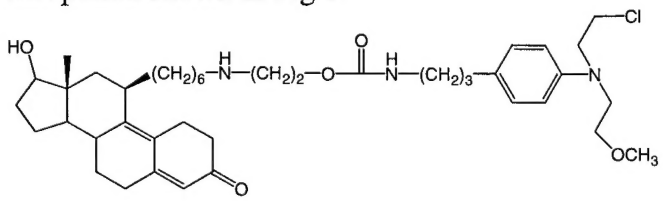

Figure 1 Monochloro 11β

We have investigated the cytotoxic effects of the monochloro 11β compound towards LNCaP prostate cancer cells. These investigations found that monochloro 11β was much less toxic than the original compound that contained 2 chloroethyl groups. Furthermore, some of the apoptotic changes induced by the dichloro compound were not found in cells treated with the monochloro 11β analog. This result suggests that the apoptotic response is somehow triggered by bifunctional DNA adducts such as intrastrand or interstrand crosslinks that can be formed by the dichloro compound but not by the monochloro

analog. We are investigating the kinetics of formation and repair of DNA adducts in LNCaP cells formed by the dichloro and monochloro compounds (see Task 2).

We have also completed the synthesis of the 17α-methyl analog of 11β. The initial step in the synthesis of the new compound was to modify the starting compound, β-estradiol-3-methylether. A methyl group was introduced at the 17α position of estrone 3-methylether using methyllithium reagent producing 17α-methyl-β-estradiol 3-methylether. The same synthetic procedues used to prepare the original 11β compound were then employed to prepare its methylated analog.

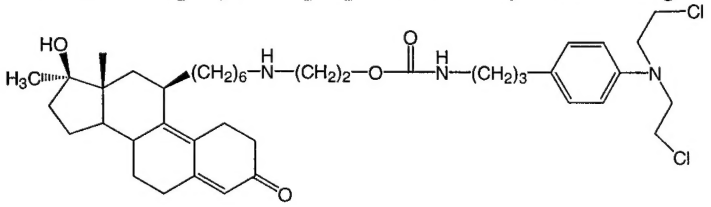

Figure 2. 17α-methyl-11β

One reason for making the new compound was to increase affinity for the androgen receptor. We found that the affinity of 17α-methyl-11β for the androgen receptor was identical to that of the original 17α-H-11β compound. Both molecules bound to the AR with affinities approximately 25% that of dihydrotestosterone. Although the affinity of the 17a-methyl was not above that of our original compound, we are evaluating its biological activities in cell culture. The presence of an alkyl group at the 17a position in other androgens has been shown to prevent metabolism and increase biological activity. During the next year will investigate whether this is also the case with our 17α-methly 11β analog.

Task 2: *Determine the fate of **11β**-DNA adducts in prostate cancer cells.*

The findings on the biological activity of the monochloro 11β analog indicate that the types and fates of the covalent DNA adducts formed by the 11β compounds are key mediators of cytotoxicity. The dichloro 11β compound can form monoadducts as well as intra- and inter-strand crosslinks in DNA, while the monochloro analog can only form monoadducts which may be easily repaired. Our results indict the DNA crosslinks formed be the 11β as the most lethal lesions and perhaps those responsible for the apoptotic response. We have begun to examine the kinetics of formation and repair of 11β-DNA adducts in prostate cancer cells. Our initial experiments used electrospray ionization mass spectometry (EIMS) to identify 11β-guanine DNA adducts in treated cells. The EIMS technique did not prove sensitive enough to accurately quantify DNA adducts in cell culture experiments. We therefore took advantage of the availability of radiolabeled [14C]-11β which has enabled us to quantify DNA adducts using the technique of accelerator mass spectrometry (AMS). AMS permits the detection and quantification of trace amounts of radioactivity. The sensitivity of this technique is over 7 orders of magnitude higher than conventional decay counting techniques. This permits us to detect low amounts of radioactivity in samples obtained from cells treated in vitro.

Fig 3 shows the results of several experiments in which we have measured the amounts of [14C]-11β that are covalently bound to DNA isolated from LNCaP cells treated for various times. We are now in the process of doing a kinetic analysis of both the formation and removal of DNA adducts in AR-expressing LNCaP cells and in DU145 and PC3 prostate cancer cells that do not express the AR. The results of these studies will determine if the presence of the AR influences the ability of cells to repair 11β-DNA lesions.

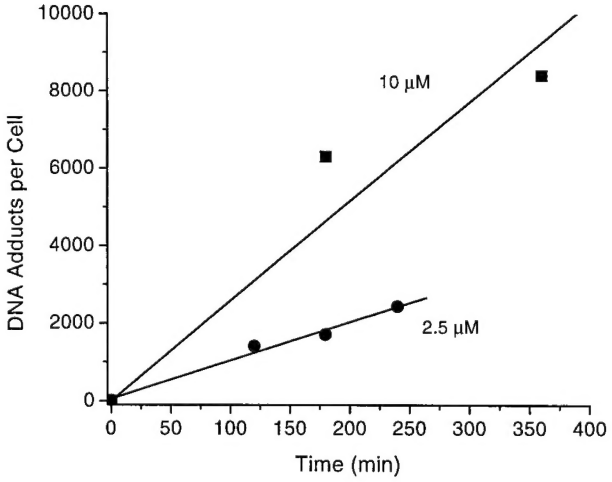

Figure 3 Formation of 11β-DNA adducts in LNCaP cells treated with [14C]-11β (—■— 10μM; —●— 2.5 μM)

Once we have a kinetic picture of the genetic damage produced by 11β we hope it will point us to the timing of key molecular changes that are responsible for apoptosis whose identify is the objective of Task 3.

Task 3: *Determine which apoptotic pathways are activated in prostate cancer cells responding to **11β**.*

We have begun to investigate the pathways and mechanisms that lead to apoptosis. Our initial experiments have examined the effects of caspase inhibitors on the proteolytic changes involved in the apoptosis in treated cells. Effects of the pancaspase inhibitor ZVAD.fmk on the cleavage of PARP confirm that caspase activation is responsible for its fragmentation after treatment of LNCaP cells with 11β. In the coming year we will examine the effects of more specific caspase inhibitors to identify the sequence of molecular events responsible for apoptosis. We will also use substrates to directly examine caspase activity. We plan to place more focus in this task once we have defined the kinetics of formation and repair of DNA adducts.

Task 4: *Assess the efficacy of **11β** in an animal xenograft model of human prostate cancer.*

We have tested the ability of 11β to inhibit the growth of LNCaP cells in a xenograft mouse model. Approximately 10^6 LNCaP cells were suspended in Matrigel and injected into the flanks of male NIH Swiss nu/nu mice. Shortly after tumor nodules appeared the animals were treated with multiple courses of 11β (30 mg/kg, administered via injection i.p.). As shown in Fig 4, the 11β compound was effective in preventing tumor growth. It is especially encouraging that the dose of 11β that was effective in preventing tumor growth did not show significant toxic effects in the animals. We are highly encouraged by these results.

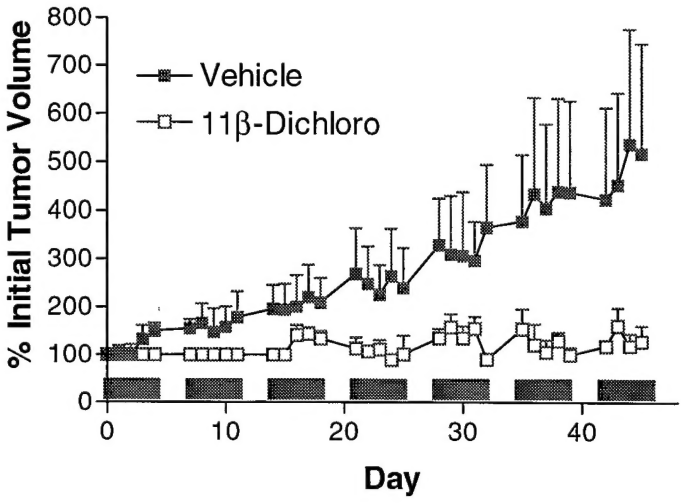

Figure 4 Effect of 11β on the growth of LNCaP xenograft tumor in NIH Swiss nu/nu mice. Animals were administered 7courses (indicated by bars at bottom) of 5 daily doses of 30 mg/kg 11β (i.p. injection) daily for 5

During the next year we will continue our mouse xenograft experiments to determine the effects of 11β on the growth of other human prostate tumors. We will also seek to optimize the dose and schedule of administration to enhance the antitumor responses.

KEY RESEARCH ACCOMPLISHMENTS

- Synthesis and preliminary evelauation of toxicity of the monochloro-11β and 17α-methyl-11β compounds.
- Development of a sensitive method for the kinetic analysis of DNA adducts formed in cells treated in vitro with [14C]-11β
- Demonstration of the antitumor activity of 11β in an animal model of human prostate cancer

REPORTABLE OUTCOMES

None during this period.

CONCLUSIONS

We have made substantial progress in the development of novel bifunctional compounds that have potential therapeutic value for the treatment of prostate cancer. Two analogs of our lead compound have been synthesized which will be valuable in understanding the mechanisms of antitumor activity. We are now engaged in experiments to define the amounts of DNA damage produced by the 11β compound and the fates of this damage in target and non-target cells in culture. This information will help us establish the sequence of events that lead to apoptosis. We anticipate that identification of the signaling events originating from DNA adducts will provide clues to the reasons why the 11β compound is able to trigger apoptosis while other aniline mustard compouds such as chlorambucil do not. The results we have obtained in animal models are especially significant. The ability of the 11β compound to prevent the growth of LNCaP cells in a mouse xenograft model provides evidence of the potential clinical activity of this compound. We will examine the activity of 11β in other xenograft models of human prostate cancer.

REFERENCES

None included.

APPENDICIES

No appended material included.